Viral-mediated gene therapy for spinal cord injury (SCI) from a translational neuroanatomical perspective

Repairing spinal cord injury (SCI) is one of the most challenging endeavours currently faced by neuroscientists. One promising therapeutic avenue to reverse this once considered permanent condition is gene therapy, however progress has been hampered by the anatomical intricacy of the spinal cord itself as well as by the sheer complexity of the molecular cascades of events that take place in the injured cord. In this perspective article, we will first discuss the priority for functional regeneration. We will then review the two main therapeutic strategies to offset the deleterious outcomes of SCI and facilitate axonal regeneration, namely the reduction of the inhibitory environment around the injury site and the up-regulation of genes that are instrumental to axonal regeneration. Further, we will provide evidence that the delivery of genes with neuroregenerative properties after a SCI has the potential to offset the deleterious effects of molecules that halt regeneration after a spinal cord injury. We will subsequently consider the three most common systems currently used to shuttle therapeutic genes in various animal models of spinal cord injury, i.e., systemic, intraspinal and intramuscular delivery techniques. We will argue that the intramuscular delivery system has several advantages over the other gene delivery methods including that it is minimally invasive and does not trigger an immune response in the spinal cord. We will then reflect on some of the problems associated with the permanent transgene expression in the central nervous system and will discuss the use of transiently expressed vectors as sound alternatives for gene therapy.

The priority for functional regeneration: The overarching goal for people with spinal cord injury, clinicians as well as regenerative neuroscientists is for functional recovery after a SCI. For functional recovery to take place after spinal cord injury, the injured axons must re-establish synaptic connections with motor neurons below the level of the trauma. In this regard, the elongation of injured axons can no longer be deemed a success if the growth is aberrant, and ultimately, non-functional. Therefore, for the re-establishment of connectivity between growing lesioned axons and neurons, target-directed synaptogenesis is absolutely critical. For example, for the return of motor function, novel synapses need to form between the interrupted descending motor tracts and the motor and/or interneurons that they once innervated. In this context, if one could up-regulate the expression of therapeutic proteins such as neurotrophic factors (NTFs) in these motor neurons, transsecting descending axons could then possibly navigate their way toward this new source of tropism. NTFs can also work through both autocrine and paracrine mechanisms and encourage synaptic formation onto the disrupted targets (i.e., motor neurons below the injury). As injured axons retain their sensitivity to NTFs, they also retain their ability to regenerate. Indeed, the growth cone tips of injured axons can be an terogradely supplied from neuronal somata with important organelles and structures, such as mitochondria, neurofilaments, and receptors to support both the elongation and synaptic processes.

What works best: reducing inhibition or promoting regeneration? One of the great debates in the field of spinal cord repair is what is the best approach to facilitate successful and functional regeneration after a SCI: reducing the inhibition surrounding SCI or promoting regeneration? The first method aims to remove/reduce inhibitory molecules that are recruited at and around the injury site and that constitute an impediment to fibre regeneration (but see Anderson et al., 2016). These inhibitory molecules include chondroitin-sulphate proteoglycans in the astrocytic glial scar as well as the myelin-associated inhibitory molecules such as Nogo, myelin-associated glycoprotein and oligodendrocyte myelin glycoprotein. The inhibition of these molecules has been shown to promote axonal elongation in the injured spinal cord, however this approach does not provide target-directed synaptogenesis. The second strategy aims to potentiate the inherent regenerative potential of injured neurons by the target-derived, up-regulation of growth-promoting protein expression such as NTFs. NTFs are known to up-regulate a family of endogenously expressed proteins that are abundant during central nervous system (CNS) development and that are essential to promote neuronal survival, synaptogenesis, axon growth and guidance as well as dendritic growth and pruning. In the adult CNS, NTF expression is mainly involved in the regulation of neuronal survival, synaptic stabilization and plasticity. NTF treatment after SCI is able to weigh the inhibitory effects of both chondroitin-sulphate proteoglycans and myelin-associated inhibitory molecules, resulting in the promotion of axonal growth through a lesion cavity (reviewed in Morris, 2014). Indeed, injured axons have been described as healthy, dynamic structures that are confined into the harsh environment surrounding the injury site. In this environment, however, injured axons can be rescued and elongate with the delivery of NTFs alone or together with other form(s) of treatment (reviewed in Morris, 2014).

Viral-mediated delivery routes for therapeutic genes: There are several ways to deliver therapeutic genes, including NTFs, to the spinal cord. Current gene transfer strategies mainly include 1) injections of viral vectors expressing therapeutic transgenes directly into the spinal cord, 2) implantation of cells genetically modified (e.g., fibroblasts, stem cells, etc.) to express particular gene(s) into the cavity created by the lesion and 3) delivery through an intrathecal catheter. While these delivery techniques result in significant transgene expression, one obstacle that hampers translation in their inherent invasiveness. Furthermore, these techniques do not encourage target-directed synaptogenesis between transected axons and motor neurons. For example, in vivo gene transfer by direct injection of viral vectors into the spinal cord has been shown to trigger a non-specific immune response (e.g., Liu et al., 1997). Likewise, implants of cells genetically modified to express a therapeutic transgene can result in immune-mediated rejection of the implants if such cells are autografts. It has also been reported that viral constructs containing the gene sequence for nerve growth factor (NGF) directly injected in the spinal cord leads to extensive sprouting of nociceptive axons that results in hyperalgesia (Romero et al., 2000), therefore compromising translation in clinical trials. Another critical drawback of these direct gene transfer approaches is that they do not promote reconnection of transected axons with their former post-synaptic targets (i.e., motor neurons and/or interneurons) and therefore are lacking functional relevance.

Intramuscular injections of viral vectors, on the other hand, are minimally invasive and therefore constitute a more clinically relevant way to shuttle therapeutic genes to the spinal cord. Intramuscularly delivered viral vectors are taken up at the neuromuscular junction by the pre-synaptic axon and are retrogradely transported along the peripheral nerves into the corresponding spinal cord motor neurons where the transgenes of interest are expressed. Over the last years, we have mapped the motor end plate (MEP) region for the main muscle groups of the forelimb and hindlimb in the rat (Tosolini and Morris, 2012; Mohan et al., 2015a) as well as in the mouse (Tosolini et al., 2013; Mohan et al., 2014) and have published a methods article that demonstrates how to locate the MEP regions in these laboratory animals (Mohan et al., 2015b). Using these maps to guide injections of retrograde neuronal tracers along the full length of the MEP regions leads to the labelling of a maximum number of central axons from injured neurons that span more spinal cord segments than previously demonstrated. For instance, with regard to the rat forelimb, targeting the MEP region for acromiotrapezius, a large muscle of the shoulder, and for triceps brachii, an elbow extensor muscle, results in the labelling of motor neurons spanning C6–C8 and C7–C8 regions of the cervical cord, respectively.

One recurrent problem with intramuscular injections of viral vectors is the resulting poor functional outcomes in animal models of SCI. In this regard, examination of the literature on intramuscular viral vector delivery reveals important methodological differences between protocols. Such differences include the types of viral vectors, viral titres, transgenes, promoters and the sites of delivery on skeletal muscle. In various reports, the forelimb muscle of the rat was routinely targeted for intramuscular injections. The MEP region, however, is rarely confined to the muscle belly. We have demonstrated that the MEP region takes different shapes and traverses the entire
The main viral vectors used in gene therapy for spinal cord injury (e.g., adenovirus, AAV, and lentivirus) are able to mediate the expression of therapeutic genes. However, adenovirus, AAV, and lentivirus can lead to significant axonal growth without triggering the side effects. For example, while brain-derived neurotrophic factor (BDNF) expression in motor neurons promotes neuroprotection (reviewed in Morris, 2014) and axonal regeneration it can also induce neuropathic pain when permanently up-regulated in dorsal root ganglia (Uchida et al., 2013). A study conducted by Liu et al. (1997) has established that direct spinal cord injections of a viral vector trigger a significant central immune response. Preliminary data from our laboratory indicate that intramuscular injections of adenovirus causes T-cell and macrophage infiltration in the skeletal muscle but not centrally in the spinal cord.

Viral vectors achieving permanent and transient transgene expression: The main viral vectors used in gene therapy for spinal cord injury are adenovirus, AAV, and lentivirus. Each of these viruses has its own unique set of appealing characteristics including the size of the gene insert, the duration of transgene expression and the ability to transduce dividing (e.g., glia) and non-dividing cells (e.g., neurons). Importantly some vectors such as AAV and lentivirus result in the permanent expression of the transgene of interest whereas adenoviral vectors only express the transgene in a transient fashion. Permanent transgene expression is required in some instances, such as for the replacement of mutant protein-coding genes that lead to genetic disorders (e.g., severe combined immunodeficiency X1). Spinal cord injury, on the other hand, is not a genetic disorder. In this context, long-term BDNF transgene expression has been shown to induce plasticity and hyper-excitability (Fouda et al., 2013). Instead, adenovirus, a transiently expressing viral-vector, would be more suitable for the short-term expression of therapeutic transgenese.

Adenovirus is double-stranded linear DNA that does not integrate into the host genome, has a large transgene capacity size (~8 kb) and is expressed transiently (e.g., days to weeks). Adenovirus enters the host cells through receptor-mediated endocytosis after binding to coxsackie and adenovirus receptors (CAR) and members of the integrin receptor family. Once internalised, adenovirus lyses the endosomal membrane, interacts with cytoplasmic dynein and is retrogradely transported along the microtubules to the nucleus where the viral-capid interacts with the nuclear envelope to induce gene expression. In our hands, when administered intramuscularly transgene expression achieved with adenoviral vectors peaks at seven days post-delivery and fades after eleven days (unpublished data). Given the fact that the growth rate of axons is estimated to be 1 mm per day (Steward et al., 2003), this time frame of expression can lead to significant axonal growth without triggering the side effects observed with vectors resulting in permanent gene expression. Moreover, adenoviral vectors, if delivered intramuscularly, would allow for the regulation of both the spatial and temporal expression of the therapeutic transgenes of interest, therefore providing a way to control the dynamics of gene therapy that cannot be achieved with other delivery methods of viral vectors that lead to permanent protein-coding gene expression.

Closing remarks: Repairing the injured spinal cord is one of the biggest challenges in the area of neuroscience. In this regard, gene therapy is certainly the most promising approach toward this goal. Indeed, the majority of therapeutic interventions aimed at reversing the deleterious outcomes of spinal cord injury have involved viral-mediated transgene delivery. A number of factors, however, need to be considered when selecting a particular experimental design, including the transgene delivery method and the choice of viral-vector. In recent years, the use of adenoviral vectors has decreased in favour of viral vectors that lead to permanent expression of the transgene(s) of interest. Likewise, intramuscular injections of viral vectors, a minimally invasive and clinically relevant way to modulate the spatial regulation of transgene expression in the spinal cord has been overlooked. It is our opinion that, with the use of fundamental anatomical knowledge of the neuromuscular system, this advantageous delivery method can lead to positive functional outcomes.

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References